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Combined Effect of Sequestration and Bioremediation in Reducing the Bioavailability of Polycyclic Aromatic Hydrocarbons in Soil

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A study was conducted to determine the combined effect of sequestration and laboratory-scale bioremediation on the bioavailability of polycyclic aromatic hydrocarbons in soil. After the compounds had aged for 140−203 days in soil, bacteria capable of degrading the compounds were added, and the availability of the hydrocarbons after bioremediation was determined. Aging decreased the amount of phenanthrene, anthracene, fluoranthene, and pyrene available to bacteria as shown by increases in the amount of the compounds remaining after bioremediation and to earthworms (*Eisenia foetida*) as shown by lower tissue concentrations, percentages assimilated, and bioconcentration factors. Aging also diminished the availability of anthracene to wheat and barley. Bioremediation caused a marked diminution in the amount of phenanthrene, fluoranthene, and pyrene taken up by earthworms. The smallest amounts of these three compounds were assimilated from soil in which they had aged and then been subject to biodegradation. The results show that the combined effects of sequestration and bioremediation lead to a more marked reduction in bioavailability than either process alone.

Introduction

Organic compounds that persist in soil undergo a poorly understood process that results in declining availability to microorganisms (*1*−*3*). This apparently results from a process by which the molecules become sequestered, although the compounds can still be recovered by vigorous solvent extraction. The sequestration is also manifested by a declining availability to higher organisms, which may be evident by diminished availability to earthworms of phenanthrene and atrazine (*4*), reduced toxicity of DDT and dieldrin to insects (*5*), or diminished toxicity of simazine to corn (*6*).

Engineered bioremediation often results in removal of much but not all of the hydrocarbons in a treated soil (*7*). Similarly, intrinsic bioremediation or natural biodegradation frequently destroys a large percentage of the compounds of concern, but a small percentage remains (*8*). It is possible that the time-dependent sequestration that renders a compound less bioavailable is the cause of the inability of the microorganisms responsible for an engineered bioremediation or natural biodegradation to destroy all of the chemical. If so, then the fraction of a compound that remains after bioremediation, although evident by vigorous extraction, may be less available to higher organisms, and thus the amount of chemical assimilated as a result of exposure to such a contaminated soil would be reduced.

This investigation therefore was designed to determine the extent to which polycyclic aromatic hydrocarbons that had been aged, or undergone time-dependent sequestration, and then been subject to simulated bioremediation in the laboratory are available to earthworms and plants.

Materials and Methods

Aging. Lima loam (pH 7.1, 11.4% organic matter) from Aurora, NY, was passed through a 2-mm mesh sieve, air-dried, and sterilized with 2.5 Mrad of γ-irradiation from a ^{60}Co source. Anthracene (98% purity, Sigma Chemical Co., St. Louis, MO), fluoranthene (99% purity, Sigma), or pyrene (99% purity, Aldrich Chemical, Milwaukee, WI) in 50 mL of methylene chloride (50 mg of test compound) was added to 500 g of sterilized soil in a sterilized glass dish, the soil was mixed thoroughly, the dish was covered with Al foil, and the solvent was allowed to evaporate. Sterile deionized water was then added to bring the moisture level to approximately 80% of its field capacity. The soil was stirred with a sterilized spatula, and the soil samples were aseptically transferred to sterile screw-capped test tubes or flasks which were almost completely full with the soils to minimize losses by volatilization. The vessels then were sealed with sterile screw caps fitted with silicone-backed Teflon liners. A soil sample was taken immediately for Soxhlet extraction to determine the initial chemical concentration. The soils were kept in the dark at 22 ± 1 °C for up to 203, 147, and 133 days for anthracene, fluoranthene, and pyrene, respectively. Aging was initiated at preselected dates before conducting the bioassays so that, for each compound, all bioassays for the various aging times were done on the same date. After the chemicals had aged, triplicate samples were used for Soxhlet extraction, mild extraction by organic solvents, measurements of earthworm and plant uptake, and moisture determinations.

Laboratory−Scale Bioremediation. Bacterial consortium PAH01 was isolated by enrichment culture using an inorganic salts solution amended with anthracene (100−200 μg/mL) and inoculated with activated sludge. Five serial transfers were made every 7 or 14 days. *Mycobacterium* sp. was provided by Richard Bartha (Rutgers University, New Brunswick, NJ). When inoculated into sterile soil containing freshly added chemicals, the microorganisms were found to be able to utilize the test compounds. For this purpose, the soil was incubated for 28 days at 21 ± 1 °C in the dark, and analysis was done by HPLC following Soxhlet extraction using methylene chloride.

To prepare the inoculum for bioremediation, the bacteria were grown for 10−14 days in a medium containing 100 mg of the hydrocarbon, 0.8 g of K_2HPO_4, 0.2 g of KH_2PO_4, 0.2 g of $FeCl_3 \cdot 6H_2O$, and 0.1 g each of NH_4NO_3, $MgSO_4 \cdot 7H_2O$, and $CaCl_2 \cdot 2H_2O$ per L. The cultures were incubated in the dark at 30 °C on a rotary shaker operating at 100 rpm for 10−14 days, by which time crystals of hydrocarbon were no longer visible. The cells were collected by centrifugation at 8500*g* for 10 min, washed at 4 °C with a solution containing 2.0 g of NH_4NO_3, 0.8 g of K_2HPO_4, and 0.2 g of KH_2PO_4/L, and resuspended in the same solution. A 3.5-mL portion of the cell suspension containing 10^6−10^7 cells/mL was inoculated into each flask containing 75 g of hydrocarbon-amended

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TABLE 1. Effect of Aging on Phenanthrene Mineralization and Its Uptake by Earthworms Before and After Bioremediation

time of aging (days)	extent of mineralization (%)	concn in soil (μg/g)		earthworm uptake			
				tissue concn (μg/g)		% assimilated	
		unrem[a]	remed[b]	unrem	remed	unrem	remed
0	72A[a]	9.78A	0.21A	45.5A	0.99A	7.16A	33.2A
14	55B	9.48B	0.80B	4.07BCD	0.032B	2.02BCD	0.20B
84	21C	9.86A	0.58B	3.19CD	0.15B	1.42CD	1.31B
126	20C	9.56B	0.66B	3.20D	0.044B	0.87D	0.10B
175	27C	10.2C	0.62B	5.17B	0.060B	2.48B	0.42B

[a] Unremediated soil. [b] Soil after bioremediation. [c] Values in columns followed by the same letter are not significantly different ($P < 0.05$).

soil at a moisture content of about 85% of water-holding field capacity. Consortium PAH01 and *Mycobacterium* sp. were inoculated into pyrene-amended soil. The soils were incubated at 22 ± 1 °C for 35, 33, and 28 days for anthracene, fluoranthene, and pyrene, respectively. The concentrations of the compounds remaining were determined at weekly intervals by Soxhlet extraction with methylene chloride and HPLC analysis. Bioremediation was considered essentially complete when the concentrations in successive analyses did not decrease further. The soil samples were then used to determine bioavailability of the test compounds.

Availability to Animals and Plants. Initial studies showed that uptake of the test compounds (1−100 μg/g) by earthworms, wheat, and barley was significantly correlated ($r^2 > 0.96$) with their concentrations in soil (Tang and Alexander, unpublished data). Therefore, earthworms and these plants were used to assay bioavailability.

Adult red worms, *Eisenia foetida* (obtained from Carolina Biological Supply Co., Burlington, NC), were introduced into soil containing PAHs aged for varying periods. Each tube contained 10 g of unremediated soil or 20 g of bioremediated soil and received eight worms. For each compound, the average weight of worms added to the tube was not significantly different ($P < 0.05$). The lower part of the tubes was covered with Al foil, and the tubes were stored in the dark at 21 ± 2 °C for 10 days.

To measure the availability of anthracene to plants, wheat or barley seedlings were aseptically transferred to sterile test tubes (38 × 200 mm) each containing 10 seedlings and 20 g soil in which anthracene had been aged for various times. The tubes were incubated in a growth chamber providing 950 lx and a 16 h/8 h light-dark cycle at 25 (light) and 20 °C (dark). The plants were harvested after 14 days and analyzed.

Analysis. Extraction and analysis of soil, six earthworms, or approximately 3 g of plants amended with 20 μg of the test chemicals in 1.0 mL of methanol, after homogenization of the samples, gave recoveries of 90−105%.

For the analysis of soil, a 1- or 2-g sample was transferred to a cellulose extraction thimble (25 mm × 80 mm). For the analysis of the earthworms, the worms were kept on wet filter paper for 24 h to allow for depuration. The worms then were washed with water, placed in liquid N_2 at −15 °C, and then weighed. After grinding with 25−30 g of anhydrous Na_2SO_4, the tissue was placed in an extraction thimble. For the plant analyses, the soil was washed from the roots, and the roots then were washed with methanol. The plants were dried on a paper towel, weighed, and cut into 1−2 mm segments, which were then ground in liquid N_2. The soil, worm, or plant tissues then were subjected to Soxhlet extraction with 100 mL of methylene chloride (HPLC grade) for 6−8 h at a rate of 5−6 min/cycle. The extracts were concentrated in a rotary evaporator at 45 °C to near dryness, the residue was dissolved in 5 mL of methanol, and this solution was passed through a 0.22-μm filter (Micron Separations, Inc., Westboro, MA) to remove particulate matter prior to HPLC analysis.

The extracts were analyzed using a Hewlett-Packard high performance liquid chromatograph (series 1050) fitted with a Spherisorb ODS-2 octadecyl-bonded silica column (Hewlett-Packard Co., 5 μm, 250 × 4 mm). Acetonitrile−water (90:10) was the mobile phase at rate of 1.0 mL/min. The compounds were detected by their UV absorbance at 250 nm for anthracene and at 240 nm for fluoranthene, pyrene, and phenanthrene.

Phenanthrene Aging. In preliminary tests, phenanthrene was added to sterile samples of Lima loam (pH 7.34, 7.7% organic matter) to give 10 μg/g. The soil was either contained in 50-mL screw-cap test tubes (10 g soil) and received 200 000−400 000 dpm of [9-^{14}C]phenanthrene (8.3 or 13.1 mCi/mmol, >98% purity, Sigma) for measurements of mineralization or in 118-mL glass jars (50 g soil) with unlabeled chemical for determination of uptake by eight or nine earthworms. Bioremediation was accomplished with a phenanthrene-degrading bacterium (*8*) grown for 4−7 days in an inorganic salts solution containing that hydrocarbon (50 mg/L), the soil initially receiving 10^6−10^7 cells/g. The $^{14}CO_2$ released in mineralization after 30−50 days, by which time little additional $^{14}CO_2$ was being produced, was trapped in 0.5 N NaOH, and the radioactivity was determined with a liquid scintillation counter.

Phenanthrene was extracted from soil in the tubes by successive *n*-butanol extractions involving 2-min shaking at room temperature and then at 30 °C and 1−4 days of shaking at 30 °C and then at 40 °C, followed successively by methylene chloride and hexanes. It was recovered from soil in the jars and from earthworm tissue by Soxhlet extraction for 6 h with a mixture of 110 mL of hexanes and 3−5 mL of butanol. Analysis was either done by determining ^{14}C if the soils had not been bioremediated or by HPLC using acetonitrile (86:14) at a flow rate of 0.8 mL/min.

Results

Preliminary tests were conducted with phenanthrene. More than 94% or more was recovered by vigorous extraction after aging periods up to 25 weeks.

The decline in bioavailability to earthworms as a result of aging occurred chiefly in the first 14 days as shown by the assimilation of 7.16% of the phenanthrene initially added and only 2.02% after 2 weeks, but there was little difference in amount assimilated thereafter (Table 1). On the other hand, the period of decline in bioavailability to the bacterium lasted for more than 14 days because mineralization continued for longer periods.

Although incubation with the bacterium was sufficiently long that $^{14}CO_2$ evolution from the radioactive substrate had largely ceased, some phenanthrene remained in the soil. More than 90% had been degraded, regardless of aging time, however (Table 1). Nevertheless, more remained in the soil in which the compound had aged than in soil to which the chemical was added immediately before inoculation. After aging, ca. 3 times more of the compound remained after

TABLE 2. Effect of Aging on Anthracene Uptake by Earthworms Before Bioremediation and Its Concentration in Soil Before and After Bioremediation by Consortium PAH01

time of aging (days)	concn in soil (μg/g)		earthworm uptake[a]		
	unremed	remed	tissue concn (μg/g)	% taken up[b]	BCF[c]
0	109.9A	0.88A	109.1A	32.2A	1.84A
29	110.1A	1.49B	86.5B	20.5B	1.29B
77	104.7A	2.18C	82.1BC	18.0BC	1.57B
147	90.5C	2.05C	74.1C	17.1BC	1.19BC
175	95.7B	2.21C	63.6D	15.5C	1.00C
203	98.8B	2.50C	65.9D	13.7C	0.99C

[a] Uptake data in soil before bioremediation. [b] Percentage of initially added compound found in the worms. [c] Bioconcentration factor.

biodegradation than in freshly amended soil that was bioremediated.

After phenanthrene had aged, the soils were subjected to bioremediation, and measurements were made of the amounts of the compound remaining after bioremediation that were assimilated by the worms. Some phenanthrene was still available to the animals. After biodegradation of the unaged compound, 33.2% was assimilated by the worms. In contrast, after biodegradation of the compound that had aged for 14−175 days, <1.4% was taken up. This decrease in uptake of the aged compound is particularly marked considering that ca. 3-fold more of the compound remained after bioremediation of the aged than unaged phenanthrene. The data also show that bioremediation does not remove all of the biologically available phenanthrene, because the animal tissues contained the compound even after much or all of that available to bacteria was destroyed.

Calculations were made of the bioconcentration factor (concentration in the organism divided by the concentration in the soil). Before bioremediation, the value was 4.90 in soil containing unaged phenanthrene, and the value fell to 0.41 after 14 days of aging and remained essentially unchanged for the test period. After bioremediation, the value was 7.07 in soil with unaged phenanthrene, and it fell to values <0.30 with soil containing the compound aged for all time periods.

Anthracene was aged for up to 203 days, and >90% of the compound could be recovered by Soxhlet extraction after the various aging periods (Table 2). The laboratory-scale bioremediation by consortium PAH01 was highly effective, and >99% of the hydrocarbon was destroyed microbiologically. Nevertheless, more of the compound remained after bioremediation of soil containing the aged compound, and the effect of aging on bioavailability reached a maximum after 77 days. The influence of aging was evident in unremediated soil, as shown by the progressively lower concentrations found in the animal tissues, and progressively lower percentages of chemical added were assimilated with the increasing aging time. The same time-dependent change is evident in the bioconcentration factors. A significantly greater degree of sequestration was not evident at the last few time points. Because of the extensive biodegradation by the microorganisms, the quantity present in the worm tissues (and in the plants, which are discussed below) was below the detection limit, which is 0.01 μg/g.

Aging also reduced the availability of anthracene to the two plant species. This is evident in tissue concentrations, percentages taken up, and the values for BCF (Table 3). The effect was detectable in wheat even after 29 days but was evident in barley by two of three parameters only after 147 days.

The availability of aged fluoranthrene to earthworms before and after bioremediation was also determined. Essentially all of the compound was recovered from soil even after 140 days of aging (Table 4). Although >98% of the freshly added hydrocarbon was destroyed by bioremediation, the amount that remained after microbial action progressively increased as the aging time increased for the full 140-day period. The availability of the compound to earthworms—as determined by tissue concentration, percentage of the compound assimilated, or bioconcentration factor—in soil not subject to biological treatment declined in the first 75 days, but additional aging did not cause a further decline in availability. Bioremediation markedly reduced the amount assimilated by the worms regardless of aging as determined by each of the three parameters, the concentration in the animals falling by more than 2 orders of magnitude as a result of the remediation. After bioremediation, less of the aged than of the unaged compound was assimilated, this decline proceeding for 75 days of aging when evaluated from tissue levels and bioconcentration factors and for at least 140 days when evaluated by percentage uptake. The values in Table 4 for percentage assimilated in unremediated and remediated soil are calculated from the amounts of anthracene added to soil and the amounts remaining after bioremediation, respectively.

TABLE 3. Effect of Aging on Anthracene Uptake by Plants

time of aging (days)	uptake by wheat			uptake by barley		
	tissue concn (μg/g)	% taken up	BCF	tissue concn (μg/g)	% taken up	BCF
0	5.29A	0.91A	0.071A	3.55A	0.58A	0.052A
29	4.40B	0.77B	0.059B	3.38A	0.49A	0.043B
77	3.76C	0.71B	0.047C	3.50A	0.50A	0.046B
147	4.32B	0.77B	0.058B	2.61B	0.37B	0.035C
175	4.26B	0.71B	0.056BC	2.35B	0.34B	0.036C
203	3.76C	0.62C	0.052BC	2.41B	0.35B	0.031C

Pyrene was studied in a similar fashion, but two cultures were used separately for the laboratory-scale remediation. As with the other compounds, pyrene became sequestered with time so that more of the aged than of the unaged hydrocarbon remained after biodegradation (Table 5). In unremediated soil, aging resulted in a progressive decrease in availability to earthworms, the values of all three parameters falling with increasing periods of persistence. Regardless of aging time and inoculum, bioremediation reduced the concentration that was found in tissues of the animal, the percentage of the compound that was assimilated, and the values for bioconcentration factors. Furthermore, the progress of aging resulted in smaller percentages assimilated and lower bioconcentration factors following biodegradation brought about by both cultures, although this effect was small or not statistically significant as determined by tissue concentrations.

Discussion

Bioremediation has been shown to reduce the toxicity of contaminated soil. For example, a reduction in toxicity to *E. foetida*, *Brassica rapa*, and *Avena sativa* was found following bioremediation of a soil contaminated with a mixture of polycyclic aromatic hydrocarbons (*9*). Similarly, bioremediation of a heavy, medium, and light oil added to soil resulted in a loss of their toxicity to *E. foetida* and germinating seeds (*10*). Other studies have shown that bioremediation of oils added to soil led to a loss of phytotoxicity (*11*), and diminished toxicity to *Photobacterium phosphoreum* of aqueous extracts of a soil amended with individual chlorophenols was noted as a result of microbial degradation of the added compounds (*12*). Declining bioavailability to *E. foetida* of phenanthrene and atrazine (*4*) and toxicity to three insect species of dieldrin and DDT (*5*) were also observed as a result of aging in soil.

TABLE 4. Effect of Aging and Bioremediation by Consortium PAH01 on Fluoranthrene Uptake by Earthworms and Its Concentration in Soil

time of aging (days)	concn in soil (μg/g)		uptake by earthworms					
			tissue concn (μg/g)		% taken up		BCF	
	unrem	remed	unrem	remed	unrem	remed[a]	unrem	remed
0	102.4A	1.65A	125A	0.36A	38.2A	7.81A	2.34A	0.27A
75	91.7B	4.56B	83.7B	0.24B	26.0B	1.89B	1.35B	0.06B
107	89.3B	5.85C	82.8B	0.30AB	25.7B	1.85B	1.30B	0.06B
140	96.0B	7.72D	87.4B	0.26B	27.1B	1.21C	1.50B	0.04B

[a] Percentage of the compound remaining after bioremediation that was assimilated.

TABLE 5. Effect of Aging and Bioremediation on Pyrene Uptake by Earthworms and Its Concentration in Soil

treatment	time of aging (days)	concn in soil (μg/g)	uptake by earthworms		
			tissue concn (μg/g)	% taken up	BCF
no remediation	0	93.5A	102 A[a]	37.5A	2.17A
	83	93.0A	88.9B	32.7B	1.41B
	133	89.0A	72.7C	26.7C	1.10B
remediation by bacterium PAH01	0	1.90A	0.28A	4.28A	0.16A
	83	4.84B	0.26A	1.36B	0.06B
	133	6.69C	0.25A	0.95C	0.04C
remediation by *Mycobacterium* sp.	0	1.77A	0.46A	6.79A	0.44A
	83	6.48B	0.37B	1.54B	0.07B
	133	6.84B	0.40AB	1.56B	0.07B

[a] Values in a column for any one treatment followed by the same letters are not significantly different ($P < 0.05$).

However, the combined action of bioremediation and aging on either animals or plants has not been assessed heretofore.

The results of the present study suggest that extensive biodegradation by microorganisms does not necessarily remove all of the fraction of an aged compound that is bioavailable since some uptake by worms occurred even after the laboratory-scale bioremedation. It is possible, on one hand, that longer periods of microbiological treatment might have resulted in removing all of the bioavailable hydrocarbon. On the other hand, it is also possible that a portion of a compound that is sequestered is available to different degrees to dissimilar organisms, particularly since the organisms must have some mechanism to release the compound from the sorbed and sequestered state. It may be that the mass of material that becomes sequestered should be considered as existing in two forms. One form may be unavailable to all organisms because it is physically remote and thus inaccessible. The second form may be differentially available, and its assimilation, toxicity, and/or biodegradation may depend on the properties of the species and its ability to mobilize the molecules from this nonremote location (*13*).

A common procedure to assess the outcome of bioremediation or sequestration is to measure the disappearance of the lethal effects of toxic substances (*3, 6, 10*). Although such bioassays do indeed show that changes in bioavailability have occurred, the findings presented here point to a danger if it is assumed that the disappearance of lethality denotes the absence of bioavailability. Although this may be self-evident to environmental toxicologists, it is a view that is commonly presented in evaluations of the health or ecological significance of bioremediation. The point is reinforced by the case of DDT, which is sequestered in soil (*13*) and whose lethality to insects totally disappears as a result of such sequestration (*5*), yet a portion of that insecticide was still assimilated by earthworms introduced into soil that was treated in the field with DDT more than 40 years before the bioassay was performed (Morrison and Alexander, unpublished data).

These data demonstrate that hydrocarbons not previously shown to be subject to aging do become sequestered in soil. Thus, the availability of anthracene, fluoranthene, and pyrene to bacteria and earthworms and of anthracene to wheat and barley declined as they persisted in soil. The data extend the observations on the bioavailability of phenanthrene to bacteria and earthworms (*2, 3*) and also on the effect of aging on the availability of other classes of compounds to plants (*6, 14*).

The sequestration of phenanthrene occurs rapidly in some soils (*15*). Thus, it is possible that some, or possibly all, of the polycyclic aromatic hydrocarbons that remained after laboratory-scale bioremediation of the freshly added compound is attributable to the sequestration that was taking place even as biodegradation was proceeding. Alternatively, it may be that the residual compound was still bioavailable but that more time would have been required to lower the concentration even further.

From the viewpoint of practical bioremediation, these observations are important because they demonstrate that animals assimilate a smaller percentage of target compounds from bioremediated soil in which sequestration has occurred than in which there has been no aging. It is also evident from these data that measurements of the effectiveness of bioremediation that are performed with freshly added compounds may not be appropriate predictors of the effectiveness in field soils in which the chemicals typically have aged for long periods of time.

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Literature Cited

(1) Steinberg, S. M.; Pignatello, J. J.; Sawhney, B. L. *Environ. Sci. Technol.* **1987**, *21*, 1201−1208.

(2) Hatzinger, P. B.; Alexander, M. *Environ. Sci. Technol.* **1995**, *29*, 537−545.
(3) Kelsey, J. W.; Kottler, B. D.; Alexander, M. *Environ. Sci. Technol.* **1997**, *31*, 214−217.
(4) Kelsey, J. W.; Alexander, M. *Environ. Toxicol. Chem.* **1997**, *16*, 582−585.
(5) Robertson, B. K.; Alexander, M. *Environ. Toxicol. Chem.* **1998**, *17*, 1034−1038.
(6) Scribner, S. L.; Benzing, T. R.; Sun, S.; Boyd, S. A. *J. Environ. Qual.* **1992**, *21*, 115−120.
(7) Loehr, R. C.; Webster, M. T. In *Environmentally Acceptable Endpoints in Soil;* Linz, D. G., Nakles, D. V., Eds; American Academy of Environmental Engineers: Annapolis, MD, 1997; pp 137−386.
(8) Carroquino, M. J.; Alexander, M. *Environ. Toxicol. Chem.* **1998**, *17*, 265−270.
(9) Hund, K.; Traunspurger, W. *Chemosphere* **1994**, *29*, 371−390.
(10) Salanitro, J. P.; Dorn, P. B.; Huesemann, M. H.; Moore, K. O.; Rhodes, I. A.; Jackson, L. M. R.; Vipond, T. E.; Western, M. M.; Wisniewski, H. L. *Environ. Sci. Technol.* **1997**, *31*, 1769−1776.
(11) Wang, X.; Bartha, R. *Soil. Biol. Biochem.* **1990**, *22*, 501−505.
(12) Dasappa, S. M.; Loehr, R. C. *Water Res.* **1991**, *25*, 1121−1130.
(13) Alexander, M. In *Environmentally Acceptable Endpoints in Soil;* Linz, D. G., Nakles, D. V., Eds; American Academy of Environmental Engineers: Annapolis, MD, 1997; pp 43−136.
(14) Hurle, K. *Weed Res.* **1977**, *17*, 25−32.
(15) Chung, N.; Alexander, M. *Environ. Sci. Technol.* **1998**, *32*, 855−860.

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